EFFECTS OF TURBIDITY-PRODUCING MATERIALS IN SEA WATER ON EGGS AND LARVAE OF THE CLAM (VENUS (MERCENARIA) MERCENARIA)

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The effect of suspended materials on mollusks and on the survival and growth of their larvae is of interest to biologists and of considerable importance to shellfish producers. Since several of the most important commercial species are essentially inhabitants of the shallow waters of bays and estuaries, almost all of the grounds used for the cultivation of shellfish are frequently subject to water rendered turbid by natural phenomena, such as floods and storms, and by human operations, such as dredging, bridge- and road-building, etc.

An extensive field study by Lunz (1938) during the dredging of the Intracoastal Waterway of South Carolina showed that the mortality of adult oysters in the dredged area, except where oysters were actually buried by the spoil, was no higher than in areas remote from the dredging operations. Moreover, he found no evidence that the physiological condition of the oysters, as judged by the yield of meats per bushel of oysters, was affected by the dredging operations. He also reported that the intensity of setting of oysters in an area adjacent to dredging operations did not differ from setting intensity in areas remote from such operations, and concluded (p. 134) that "dredging operations apparently had no effect on spawning and setting."

In careful quantitative experiments Loosanoff and Tommers (1948) have shown that as little as 0.1 gram per liter of silt reduced the average pumping rate of adult oysters by 57 per cent. At a concentration of 1.0 g./l. they found the reduction in average pumping rate was more than 80 per cent and reached 94 per cent when the concentrations of silt were increased to 3.0 and 4.0 g./l. They reported that results with kaolin and chalk were similar to those obtained with silt, and that 0.5 g./l. of Fuller's earth, the only concentration tested, reduced the rate of pumping by 60 per cent.

Since the development of techniques for rearing bivalve larvae in the laboratory (Loosanoff and Davis, 1950), these techniques have been used to determine quantitatively the effects of various factors, such as temperature, species of food organisms, crowding, quantity of foods and salinity on the survival and growth of bivalve larvae (Loosanoff, Miller and Smith, 1951; Loosanoff and Davis, 1953; Loosanoff, Davis and Chanley, 1953a; 1953b; Davis, 1953; Davis and Guillard, 1958; Davis, 1958). In the present study they have been employed to determine quantitatively the effect of various concentrations of several materials suspended in sea water on the development of eggs of the hard clam, *Venus* (*Mercenaria*) *mercenaria,* and on the survival and growth of their larvae.

The turbidity-producing materials used in these experiments were clay (kaolin N.F. VII Mallinckrodt), Fuller's earth (dusting powder, McKesson), chalk (precipitated chalk U.S.P. McKesson and Robbins) and silt. The silt was collected from the bed of the Wepawaug River, just upstream from our laboratory. The silt was washed through a 325-mesh, stainless steel screen (meshes approximately 50 microns square) to remove larger particles, collected in a Büchner funnel, where it was washed with distilled water to remove salt, and then dried at 200.0° C. Finally, the dried cake of silt was ground in a jar mill. In the later experiments the Fuller's earth was also ground in the jar mill. Concentrations of suspended material are, therefore, all expressed as grams of dry powder per liter.

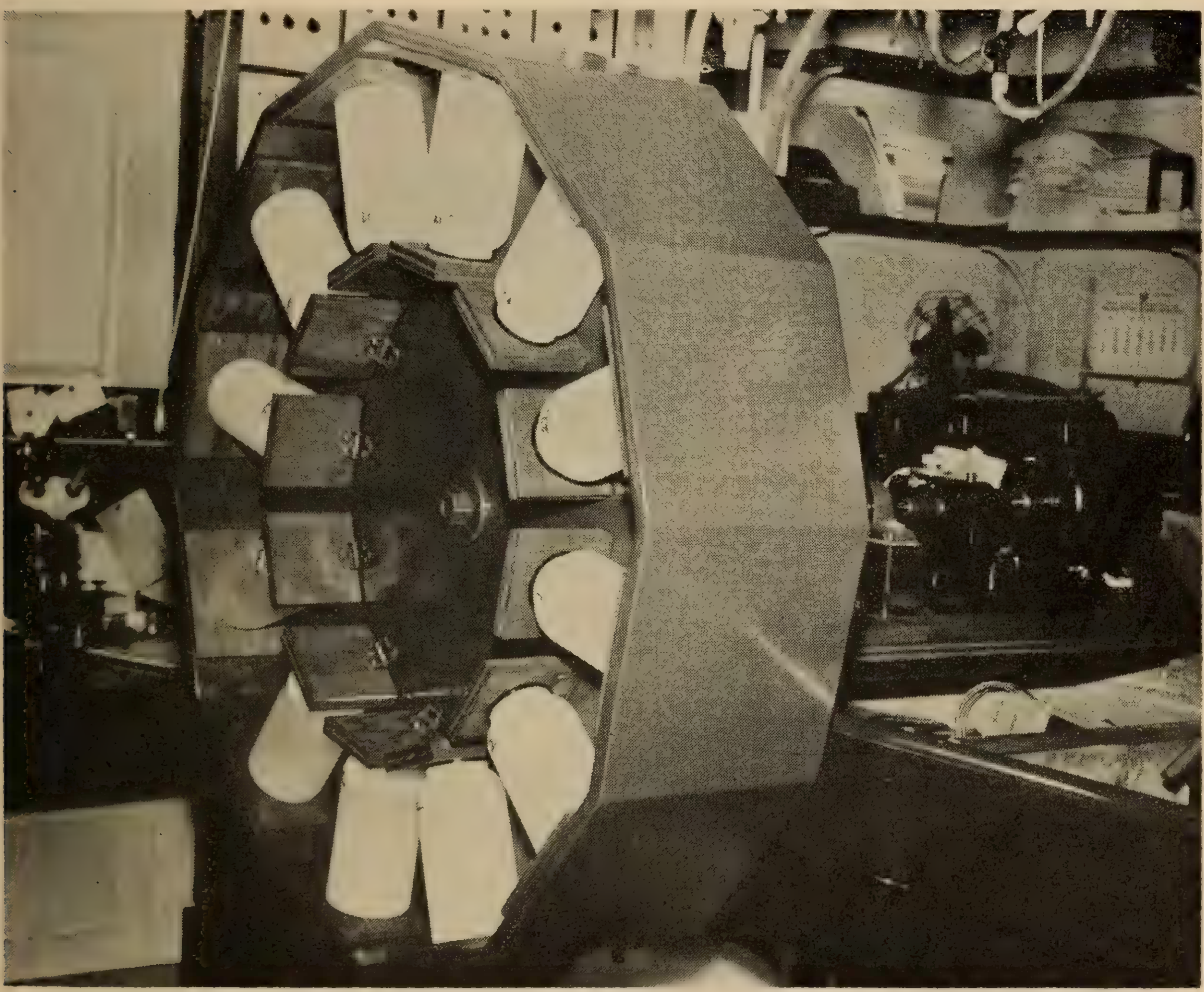

FIGURE 1. Turbidity apparatus showing the wheel and method of attachment of bottles. Motor and reduction gear box indistinctly seen in background.

In making up a suspension, a weighed quantity of the powdered material was thoroughly mixed with sea water and the various experimental concentrations prepared by serial dilution.

The method for obtaining fertilized clam eggs in midwinter has previously been described (Loosanoff and Davis, 1950) as have the methods for determining the percentage of eggs developing to the straight hinge stage, and for determining the rate of growth of larvae (Davis, 1953, 1958).

In the present series of experiments, wide-mouthed, 32-ounce polyethylene bottles with screw caps were used as containers for the larval cultures. To keep the materials in suspension we used a modification of an apparatus which V. L. Loosanoff of this laboratory observed in P. R. Walne's laboratory at the Fisheries Experiment Station, Conway, England. The bottles were mounted on a vertical wheel which, as it rotated, turned the bottles end over end once for each revolution of the wheel. In our apparatus provision is made for 12 bottles on each side of the wheel for a total of 24 bottles so that 12 sets of duplicate cultures can be run concurrently (Fig. 1). The wheel is kept rotating by a constant speed motor working through a speed reducer giving eight revolutions of the wheel per minute. The wheel and attached bottles are enclosed in a plywood box in which the air

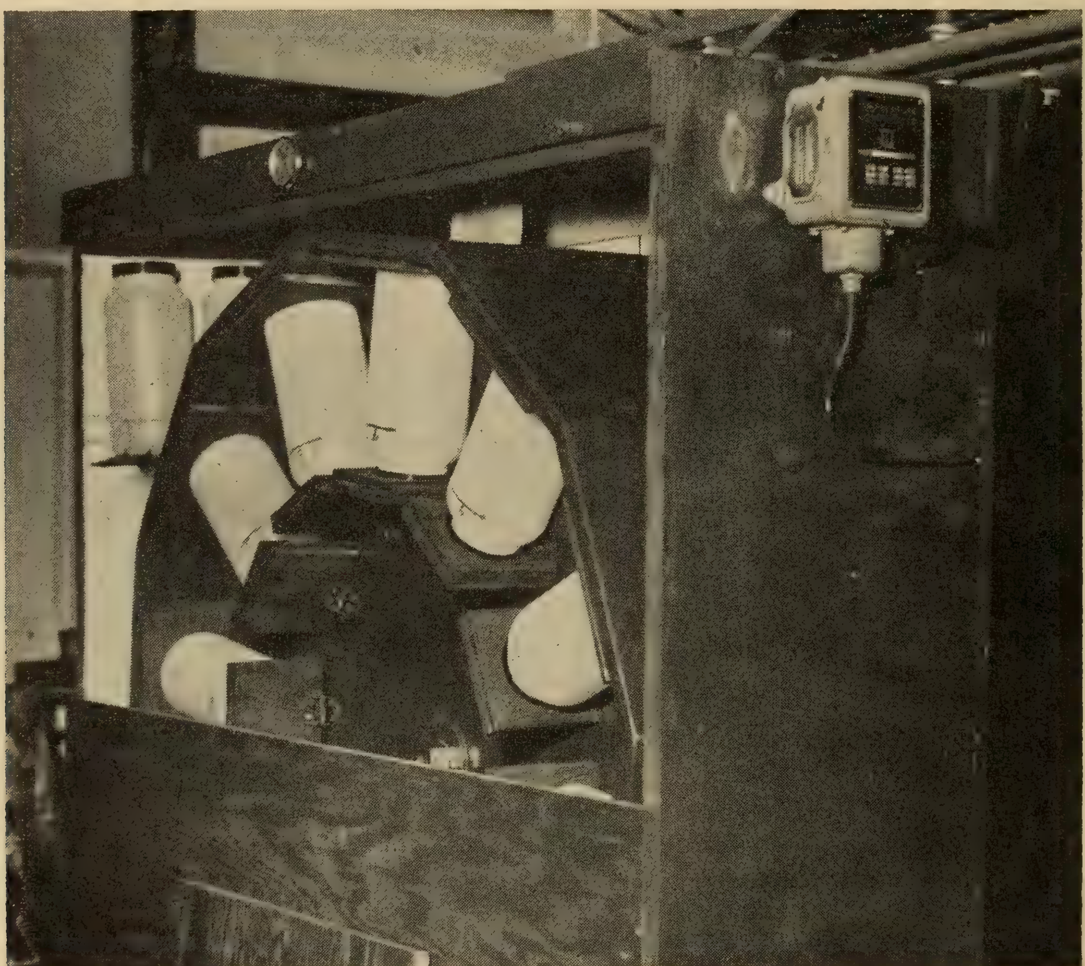

FIGURE 2. Turbidity apparatus showing thermostat control, temperature box and position of stationary control cultures.

temperature is thermostatically controlled at 24.0° C., using two 60-watt light bulbs as heaters. A wire mesh shelf is installed in the left upper corner of this box to hold two additional bottles for stationary control cultures (Fig. 2). In practice, one pair of bottles on the wheel is used for moving control cultures, leaving 11 pairs of bottles for duplicate cultures of larvae at each of 11 different sets of experimental concentrations of suspended materials.

The number of larvae suspended in the 800 ml. of sea water and turbidity-producing material in each bottle was initially the same for all cultures in an experiment, but ranged from 8000 per bottle in some experiments to about 15,000 in others. The larvae were fed daily, each culture receiving an equal quantity of a mixture of *Isochrysis* and *Monochrysis*. The larvae were examined every second day when the sea water and its suspended material were changed, but quantitative samples for counts and measurements were taken only when the larvae were 48 hours old and on the twelfth day, when an experiment was terminated.

Preliminary experiments indicated that some fertilized clam eggs could develop into straight hinge larvae in concentrations of either clay (kaolin) or chalk as high as 4.0 g./l., but that none developed to the straight hinge stage in equivalent concentrations of either Fuller's earth or silt. Subsequent experiments, however, have shown that if Fuller's earth was first ground in a jar mill, some larvae developed normally at 4.0 g./l. Similarly, ground silt at concentrations of 3.0 or 4.0 g./l. still completely prevented normal development of clam eggs (Table I). In silt concentrations of 0.75 g./l. or lower, however, there were no significant differences in the percentage of clam eggs developing normally, while with clay, chalk or Fuller's earth there appeared to be a more or less consistent stepwise reduction in the percentage of eggs developing normally with each increase in the quantity of suspended material (Table I).

TABLE I

Percentage of clam eggs developing to the straight hinge larval stage in different concentrations of suspended materials. The number of eggs developing to the straight hinge stage in the stationary controls is considered 100 per cent

Concentration g./l.	Percentages			
	Silt	Clay (kaolin)	Fuller's earth	Chalk
Stationary control	100	100	100	100
Moving control	91			
0.125	95	82	75	
0.188	90			
0.250	96	82	61	approx. 45
0.375	93			
0.500	99	52	41	
0.750	92			
1.000	79	37	57	approx. 30
1.500	65			
2.000	39*	49	50	approx. 39
3.000	0			
4.000	0	42**	45***	approx. 45****

* Averaged 195.80 μ
** Averaged 203.90 μ
*** Averaged 199.60 μ
**** Averaged 189.10 μ
At 12 days when kept in these turbidities only during the first 48 hours after fertilization and then returned to normal sea water.

Although in a silt concentration of 2.0 g./l. only 39 per cent of the eggs developed to the straight hinge larval stage, almost all of these larvae were capable of surviving and growing to metamorphosis (mean length 195 μ at 12 days) if returned to normal sea water. Similarly, larvae developing to straight hinge stage in concentrations of 4.0 g./l. of clay, chalk or Fuller's earth have been reared to setting stage after being returned to normal sea water at the end of 48 hours.

Experiments on the effect of suspended materials on the growth of clam larvae that have developed to the straight hinge stage in our usual sea water have shown that such larvae cannot grow in concentrations of clay, Fuller's earth or chalk as high as those at which some eggs developed. For example, there was no evi-

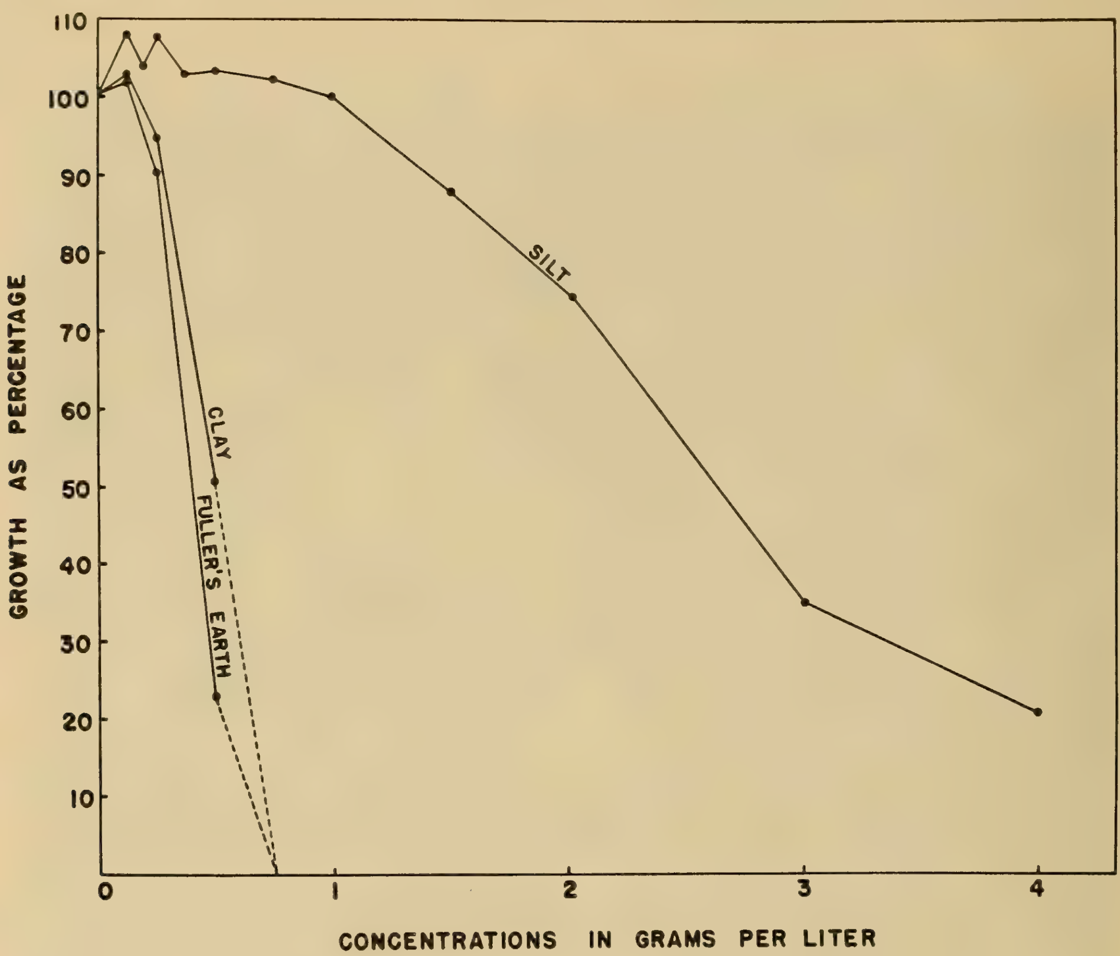

FIGURE 3. The 12-day increase in mean length of clam larvae, grown in different concentrations of suspended materials, plotted as percentages of the increase in mean length of larvae in control cultures. Each point represents the average for 50 larvae from each of duplicate cultures in each of two experiments.

dence that the larvae were taking the flagellates provided as food, and there was no growth of the larvae in a concentration of chalk as low as 0.250 g./l. With either clay or Fuller's earth, 0.500 g./l. was the highest concentration in which the larvae showed evidence of taking food (color in digestive gland) or any increase in size (Fig. 3). In cultures receiving higher concentrations of clay or Fuller's earth, many larvae ingested some of the suspended particles, apparently in sufficient quantity to block the digestive tract and cause death. Kaolin in a concentration of 0.500 g./l. caused approximately 50 per cent mortality in 12 days and almost complete mortality at all higher concentrations. There was no appreciable mortality of larvae in 0.500 g./l. of Fuller's earth in 12 days, but at all higher concentrations mortality exceeded 90 per cent.

When silt was used as the suspended material the results were quite different from those with clay, Fuller's earth or chalk. Clam larvae showed evidence of taking food (color in digestive gland) even at a concentration of 4.0 g./l. of silt, but growth was negligible, and it is doubtful if any larvae in natural waters could reach metamorphosis in either 3.0 or 4.0 g./l. of silt. Growth of most larvae was also considerably retarded by concentrations of 2.0 g./l. or even 1.5 g./l. of silt, but some larvae under these conditions had reached setting size (maximum length 185 μ and 200 μ, respectively) by the twelfth day. Growth of clam larvae was approximately normal in 0.750 g./l. of silt and, at all lower concentrations, was somewhat better than that of larvae in the control cultures (Fig. 3). There was no appreciable mortality of clam larvae, within 12 days, in any of the concentrations of silt tested.

Soil particles ranging in size from 62 microns to 4 microns are listed as silts, while particles ranging in size from 4 microns to 0.24 microns are listed as clays. From our experiments it would appear that it is the larger particles (coarse silt 62 to 31 microns) in the silt and unground Fuller's earth, or aggregates of particles in other substances that interfered with development of clam eggs, while the smaller particles, characteristic of the clays, probably had little effect on egg development. The results of experiments on growth of larvae, however, seem to indicate that the larger particles, characteristic of coarse silts, may have little effect on growth. It seems to be the smaller particles, as in clays, precipitated chalk, and finely ground Fuller's earth, which are about the size of food cells, that interfere most with growth of larvae. This interference appears to be primarily mechanical through blockage of the digestive tract, but there is some indication that it may also have been, at least in part, an indirect result of an adverse effect of the suspended materials on the food organisms.

The more rapid growth of clam larvae noted in lower concentrations of silt and at the lowest concentrations of clay and Fuller's earth is perhaps due, in part, to chelation of toxic substances, and with silt, in part, to positive growth factors. An improvement in the rate of growth of algal cultures upon addition of soil extract has long been known to botanists, and soil extract has been a basic ingredient in many of the media for algal cultures.

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Summary

1. Some clam eggs developed normally in concentrations of 4.0 g./l. of clay, precipitated chalk or finely ground Fuller's earth, although the percentage developing normally decreased as the concentration of these suspended materials increased.

2. In silt concentrations below 0.75 g./l. the percentage of clam eggs developing normally was not significantly different from that in control cultures but decreased progressively in successively higher concentrations.

3. None of the clam eggs developed normally in silt concentrations of 3.0 or 4.0 g./l.

4. Larvae resulting from clam eggs developing in high concentrations of each of the suspended materials were reared to metamorphosis after being returned to normal sea water at 48 hours.

5. Clam larvae were unable to grow in concentrations of clay, chalk or Fuller's earth as high as those at which some eggs developed.

6. The highest concentration of chalk was 0.25 g./l. and 0.5 g./l. was the highest concentration of clay and Fuller's earth at which clam larvae showed any growth and mortality exceeded 90 per cent at all higher concentrations.

7. In a silt concentration of 0.75 g./l. growth of clam larvae was approximately normal and at lower concentrations was slightly faster than that of larvae in control cultures.

8. In silt concentrations of 1.0 to 2.0 g./l. growth of clam larvae was retarded and at 3.0 and 4.0 g./l. growth was negligible.

9. Even at a silt concentration of 4.0 g./l. there was no appreciable mortality of clam larvae within 12 days.

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